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J.W. Castellani, C. Mahoney, C. O'Brien

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Tyrosine Supplementation Attenuates Cognitive & Psychomotor Deficits in Cold Environments

John W. Castellani, Ph.D.
Caroline Mahoney, Ph.D.
Catherine O'Brien, M.S.
US Army Research Institute of Environmental Medicine
Natick Soldier Research, Development & Engineering Command
15 Kansas Street
Natick, MA 01760

john.castellani@us.army.mil

ABSTRACT

In rats, dietary supplementation with the amino acid tyrosine (TYR) prevented depletion of central catecholamines observed during acute environmental stress. Concomitant changes in the animals' behavioral and cognitive responses to stress suggest that TYR might have similar effects on cognitive, psychomotor, and physical performance in humans exposed to environmental stress. This study evaluated the effect of TYR supplementation on cognitive, psychomotor, and physical performance during and following a cold water immersion that lowered body core temperature. Volunteers (n = 19 during immersion; n = 15 following immersion) completed a control trial (CON) in warm (35ºC) water and two cold water trials (10-18°C), each spaced a week apart. Subjects ingested a 285 kcal energy bar during each trial; on one cold trial (TYR) the bar contained tyrosine (300 mg / kg body weight), and on the other cold trial (PLB) and on CON the bar contained no tyrosine. During PLB, volunteers made fewer correct responses on a Match to-Sample memory measure (decreased by 2.4 relative to baseline measurements; p<0.05). When volunteers consumed TYR, correct responses increased on a Match-to-Sample (increased by 0.14 relative to baseline; p<0.05) and study time for the Match-to-Sample was shorter by 1.38 seconds (p<0.05), indicative of more rapid and accurate information processing. Following each water immersion, subjects completed a battery of performance tasks in a cold air (10ºC) chamber. Core temperature was lower (p=0.0001) on PLB and TYR (both 35.5 ± 0.6 ºC) than CON (37.1 ± 0.3 ºC). On PLB, marksmanship performance decreased 14% (p=0.002) and Match-to-Sample declined by 18%, compared to CON, but there was no difference between TYR and CON. Step test performance decreased by 11% (p=0.0001) on both cold trials, compared to CON. This study supports previous findings that cold exposure degrades cognitive performance and supplementation with TYR alleviates working memory decrements, even with a reduced core temperature. These data also demonstrate that TYR attenuates the cold-induced degradation in marksmanship, a psychomotor performance indicator.

1.0 INTRODUCTION

Stressful conditions that elevate brain catecholamine activity, such as cold or heat stress, altitude exposure, and tail shock in animals, are often associated with decreased cognitive performance (16; 18; 23; 33). One reason may be depletion of central catecholamines, since both norepinephrine (29) and dopamine (14; 45) are important for acquiring and performing cognitive and motor skills. Catecholamines serve as neurotransmitters, so augmenting the availability of the amino acid tyrosine, the precursor for catecholamine synthesis, through dietary supplementation could help maintain brain function by sustaining brain neurotransmitter levels (48). Tyrosine and other neutral amino acids are competitive binders to the transport

receptor for crossing the blood brain barrier, therefore, when there is a higher ratio of tyrosine relative to total neutral amino acids the rate of tyrosine transport will increase (12). Under stressful conditions that activate tyrosine hydroxylase, the rate-limiting enzyme that catalyzes the conversion of tyrosine to L-dopa, the enzyme becomes more responsive to increased intraneural tyrosine, whether through ingestion or injection of tyrosine (1; 12). Animal studies have demonstrated that supplemental tyrosine increases brain levels of norepinephrine (18; 23; 49) and dopamine (1).

Soldiers are required to perform missions even at environmental extremes; therefore, identification of effective countermeasures to mitigate performance degradation due to environmental stress is important. One example is the 1995 hypothermia deaths of four Ranger students, where decision-making may have contributed to prolonged cold water immersion (47). Both animal and human studies provide evidence that supplemental tyrosine is effective at limiting cold-induced decreases in cognitive performance (7; 29; 35; 49). In humans, Banderet and Lieberman (7) found supplemental tyrosine was effective at decreasing symptoms such as headaches, cold sensation, and fatigue during a combined stress of cold and hypoxia. Performance on cognitive tests, including addition, coding, map/compass, pattern recognition, and reaction time was also better with tyrosine, compared to placebo. Shurtleff and Thomas (35) found that tyrosine offset the 30% reduction in performance on a Match-to-Sample test that involves short-term working memory after 60 min cold (4ºC) air exposure. Tyrosine had no effect during a 22ºC control condition, suggesting that tyrosine availability only became important during the cold condition when there was increased firing of catecholaminergic neurons. While these studies provide evidence for the effectiveness of tyrosine supplementation in a cold environment, none of the human studies used a cold stress that was sufficient to reduce core temperature. Furthermore, no studies have evaluated the effect of tyrosine supplementation on physical performance during cold stress.

The purpose of the present study was to evaluate the effect of tyrosine supplementation on cognitive, psychomotor, and physical performance during cold water immersion and during cold air exposure following a stressful repeated cold water immersion protocol that induces mild hypothermia (9). Based on previous studies, it was hypothesized that cold-induced cognitive performance decrements would be mitigated with tyrosine. Marksmanship performance has been previously demonstrated to be sensitive to environmental stressors (37; 38), therefore, it was hypothesized that decrements in performance on this psychomotor task would also be mitigated with tyrosine. A secondary aim of this study was to identify physical performance tasks that are sensitive to body core cooling, and whether tyrosine reverses any observed physical performance decrements.

2.0 METHODS

2.1 Subjects

This protocol was approved by the U.S. Army Research Institute of Environmental Medicine Scientific and Human Use Review Committees. Written informed consent was obtained from each person who volunteered to participate after being informed of the purpose, experimental procedures, and known risks of the study. Investigators adhered to U.S. Army Regulation 70-25 and U.S. Army Medical Research and Materiel Command Regulation 70-25 on the Use of Volunteers in Research. The volunteers were enlisted Soldiers. Nineteen subjects were used for the cold water immersion portion of the study and 15 for the cold-air part of the study. The physical characteristics for the 19 subjects were age 21 ± 3 yr, height 174 ± 7 cm, weight 77 ± 11 kg, and % body fat 19 ± 6 %. Their physical characteristics for the 15 subjects in the cold-air portion were age 20 ± 2 yr, height 174 ± 7 cm, weight 76 ± 11 kg, body fat 19 ± 6 %, and VO_{2peak} 48 ± 8 ml / kg / min.

2.2 Experimental Design

Volunteers completed all three test conditions on separate days; thermo-neutral/placebo (CON), cold/placebo (PLB) and cold/tyrosine (TYR). The order of treatment in the cold was counterbalanced and there was a minimum three-day washout period between testing days(25; 28). Volunteers were instructed to refrain from alcohol, smoking, and exercising 12 hours prior to the start of each test day and could not eat or drink anything except water after 2200 hours the evening before the test day.

Each test session consisted of two, 90-minute water immersions, with a rewarming and recovery period (or rest if thermo-neutral) between immersions. During the cold treatment, the water temperature was approximately 10°C, but varied systematically according to individual percent body fat. The exact water temperature (to the nearest whole degree) for each participant was calculated using the Tikuisus Cold Water Survival Model (42) to induce a fall in core temperature from 37°C to 35°C in approximately 90 minutes. During the thermo-neutral condition, the water was 35°C.

When volunteers arrived for testing, they were instrumented with a rectal core temperature probe, skin temperature electrodes, and heart rate electrodes and then rested in a seated position for 15 minutes while baseline measurements were taken. Once baseline physiological measures were obtained, a baseline battery of cognitive tasks and questionnaires was completed. Volunteers then consumed either tyrosine or placebo. Following consumption volunteers were immersed while seated to the chest in circulated water (either 35°C or 10°C) for 90 minutes. For safety reasons, immersion was terminated before the 90 minute period was complete if the participant's core temperature fell below 35.0°C, or dropped 0.6ºC in five minutes, or the participant asked to stop testing. Following the first immersion, the cognitive tests were completed and then the volunteer began the rewarm/recovery phase. The core temperature at the beginning of the second immersion during each test day was similar to the temperature observed prior to immersion during the first trial. In order to ensure this, participants were warmed (within 0.5°C of the pre-immersion temperature) in warm water (39°C) after the first immersion. A second dose of tyrosine or placebo was given during the rewarming period. Following the rewarming period, participants completed an identical second 90 minute immersion and cognitive testing took place immediately after immersion.

2.2.1 Tyrosine or Placebo Administration

A low fat, high-energy nutrient bar was the matrix used for delivery of tyrosine. The bars were matched for taste and texture to ensure volunteers could not distinguish tyrosine from placebo bars. Volunteers received a tyrosine dose of 150 mg/kg of body weight before each immersion. Thus, each participant received a total of 300mg/kg of body weight of tyrosine over the course of the test day. Each nutrient bar contained 9.3 grams of tyrosine. The quantity of the bar given to each participant was adjusted based on the weight of the bar in grams. Participants consumed the bar and 1/2 glass of water 30 minutes before the start of each immersion.

2.3 Measurements

2.3.1 Physiological Measurements

Rectal temperature was measured by a thermistor inserted 10 cm past the anal sphincter. Heat flow disks, used to measure skin temperature, were secured on 10 sites (right side of the body): foot, calf, thigh, chest, triceps, anterior aspect of the forearm, subscapular, forehead, and hand (dorsal). Heart rate (HR) was monitored from three chest electrodes (CM-5 configuration) and radiotelemetered to an oscilloscope-cardiotachometer (Hewlett-Packard).

2.3.2 Cold-Water Immersion Cognitive Tests and Questionnaires

Volunteers were evaluated using multiple, dependent measures of symptoms, mood, and cognitive performance. Measures were administered on IBM compatible lap-top computers, except for the Environmental Symptoms Questionnaire which was administered by paper and pencil. Volunteers completed 5 practice sessions with the test battery in a thermoneutral environment to familiarize themselves with the task and minimize practice effects.

2.3.2.1 Visual Vigilance

This test of visual vigilance was designed to resemble military tasks requiring sustained scanning of the visual environment for infrequent, difficult to detect stimuli such as those during sentry duty (11). The task required the participant to detect a small, faint stimulus that randomly appeared for a second at various locations on the computer screen On the average, presentation of a stimulus occurred once a minute. The participant was told to respond as quickly as possible when a stimulus was detected. Dependent measures included correct detections and the response time. Responses made before (or after) stimulus occurrence were recorded as false alarms. The duration of this test was 20 minutes.

2.3.2.2 Four-Choice Visual Reaction Time

Volunteers were presented with a series of visual stimuli at one of four different spatial locations on the computer screen (20; 21). The volunteer's task was to indicate the correct spatial location of each stimulus by striking one of four corresponding keys on the computer keyboard. Dependent measures included the response latency for each trial, premature errors (responding before the presentation of the stimulus), errors of omission (response latency >1 sec) and errors of commission (hitting the wrong key). The duration of this test was approximately 5 minutes.

2.3.2.3 Delayed Match-to-Sample

This is a subtest of the Walter Reed Performance Assessment Battery. During this test, a sample pattern of 36 red and green grid squares was presented on the computer display. The volunteer studied this pattern and then pressed a key on the keyboard. The sample pattern disappeared and the screen was blank for either 8 or 16 seconds ("delay"). Next, two patterns were presented and the volunteer selected the pattern that matched the previous sample pattern. Dependent measures included correct responses, incorrect responses, time out errors, study time for the sample, and response time. The delayed match-to-sample test consisted of 20 trials and required about 12 minutes to complete.

2.3.2.4 Profile of Mood States Questionnaire

The questionnaire is an inventory of self-reported mood states (26). Each volunteer was asked to rate a series of 65 mood-related adjectives on a five point scale, using the response set of "How are you feeling right now?" The adjectives factor into six mood subscales (tension, depression, anger, vigor, fatigue, and confusion) (27). The POMS is a widely used, standardized, computer or paper-and-pencil administered inventory of mood states. It is the most frequently employed self-report mood questionnaire for assessing normal human mood state, having been employed in hundreds of published studies (27). It is sensitive to a wide variety of environmental factors; sleep loss, nutritional manipulations and sub-clinical doses of various drugs (7; 11; 22; 24). The POMS required about 5 minutes to complete.

2.3.2.5 Environmental Symptoms Questionnaire

The Environmental Symptoms Questionnaire was administered to assess symptoms of cold stress, as well as alertness, muscle discomfort, and distress (32). The items used to determine cold stress include statements like “My hands are shaking or trembling,” “I feel weak,” “I feel chilly,” and “I’m shivering.”

Volunteers rate each statement using a 6-point scale with discrete anchor points that vary from "NOT AT ALL" to "EXTREME." This questionnaire required about 5 minutes to complete.

2.3.3 Cold-Air Performance Battery

U.S. Special Operations Command (SOCOM) uses a standardized set of tests to evaluate cognitive (41) and physical performance (44) under a variety of stressful conditions. These tests were chosen by their relevance to mission requirements, the accuracy and reproducibility of the measurements, and the time and equipment required for administration of the tests. The performance tests were administered on each trial in a cold air (10ºC) chamber to limit rewarming, and they were completed in order of increasing metabolic activity in order to maintain reduced body temperature as long as possible. The cognitive tests were completed first, followed by weapon disassembly/reassembly, marksmanship, hand grip strength and endurance, pull-ups, and step test. Due to spatial constraints, the SOCOM marksmanship task was replaced by one that has been previously used in environmental extremes (37; 38). Five familiarization sessions were completed at a neutral room temperature for each task before testing began. A self-paced cycle ergometer test was added to the protocol after the first few subjects had completed testing. Eleven of fifteen subjects performed this task, which was practiced on three occasions before testing began. This task was not part of the SOCOM battery.

2.3.3.1 Cognitive Tests

The computer-based cognitive tests are described in detail by Thomas & Schrot (41). Match-to-Sample evaluates short-term spatial memory and pattern recognition. Upon subject initiation, an 8 x 8 matrix appears with a random pattern of red or green squares. After 3 seconds, the screen blanks for either a short (1 sec) or long (15 sec) delay, after which two matrices appear, only one of which matches the original (the other differing in one or two of the 64 squares). Time to make a response and which matrix is chosen are recorded. Completion of 20 trials takes ~5 min. Complex Reaction Time displays a set of boxes in the same orientation as the four arrow keys on the keyboard. A red square appears randomly in a box, and the subject must press the corresponding arrow key, after which the red square immediately appears in another square. Completion of 60 presentations takes ~1 min. Response time and accuracy are measured. Visual Vigilance evaluates sustained visual attention and choice reaction time. Letters or numbers appear briefly (0.5 sec) in the center of the screen, with random delays (1-5 sec) between presentations. The subject must press the down-arrow when only "A" or "3" appear. This task takes ~6 min for 100 presentations. Serial Addition/Subtraction measures the ability to perform simple calculations. Two digits are presented with either a plus or minus sign. If the answer is positive, the last single digit of the answer is to be entered. If the answer is negative, the subject must add 10 and then enter the resulting single positive number. This task takes ~2 min for 50 presentations. Logical Reasoning measures general reasoning ability using true or false statements about the sequence of two letters presented on the screen, "AB" or "BA." The statements are positive/negative and active/passive, and refer to whether one letter precedes or follows the other. This task takes ~3 min for 32 presentations. Repeated Acquisition assesses the subject's ability to learn, decode, or acquire a key press sequence. Twelve blocks are presented and the subjects must learn the sequence of up, down, right, or left arrow keys by trial and error over 15 total presentations. This task takes ~8 min.

2.3.3.2 Psychomotor Tests

Two psychomotor tasks were administered. One was disassembly and reassembly of an M-16 rifle (12 steps), with total time recorded. The other task was rifle marksmanship speed and accuracy, measured using a single stationary target laser system (Noptel, Oulu, Finland). Subjects waited with the rifle below waist level, then took their shot after a red LED light appeared at a random time (1-10 sec). Three sets of five shots fired from a standing position on a target simulating 46 cm at a distance of 50 m were analyzed. Calculated parameters included Distance from Center of Mass (DCM, mm), which is the distance between the average of a five shot series from the center of the target; Shot Group Tightness (SGT, mm^2), which is the area

in which the five shots are clustered; Horizontal SGT and Vertical SGT (mm), which represent the spread of the five shots in each direction; Horizontal Deviation and Vertical Deviation (mm), which represent the average directional deviation of the five shots from the center of the target, with a negative value indicating left or below the target; and Sighting Time (min), which is the time the red LED light appears until the trigger is pulled. This task took ~2 min.

2.3.3.3 Physical Performance Tests

The physical performance tasks are described in detail by Valaik et al. (44). Hand grip strength was measured in both hands on three maximal efforts, followed by a measure of handgrip endurance. Because tissue cooling can affect muscle strength, the hand grip endurance test used the force determined as 50% of the average maximum grip strength obtained during the last practice session (normal room temperature). Thus, even if cold stress on any trial altered maximum grip strength, grip endurance was still measured at the same absolute force. Hand grip strength and endurance took ~5 min. Pull-ups were performed from a hanging position with knees bent. The maximum number of pull-ups (full arm extension to chin over the bar) was recorded. A single step test was performed while wearing a 20 kg weighted vest. Subjects were instructed to complete as many steps (up with both feet and down with both feet counting as one step) as possible in 1 min. Immediately following the step test, subjects completed a self-paced cycle ergometer test of a fixed amount of work (3 kJ per kg body weight). The initial work rate was set for each subject to reflect a 50% VO_2 peak exercise intensity at a pedal cadence of 60 rpm, but the subjects could thereafter alter the work rate according to their preference throughout the test. Subjects were instructed to complete the task as quickly as possible. The cycle ergometer test was practiced at a neutral temperature on three occasions before testing began.

2.4 Statistical Analysis

Data were analyzed across all three trials using repeated measures analysis of variance. Tukey's Honestly Significant Difference (HSD) post-hoc test was applied when significant main effects were found. Statistical significance was set at $p<0.05$. Cold-Water Immersion cognitive data are presented as the change from baseline.

3.0 RESULTS

3.1 Core Temperature Results

The cold water immersions reduced rectal temperature ($p<0.0001$) by ~1.5ºC in both PLB (35.5 ± 0.6 ºC) and TYR (35.4 ± 0.5 ºC) trials, which were significantly different ($p<0.05$,) from CON (37.1 ± 0.3 ºC), but not from each other. During cold air exposure, mean skin temperature was lower ($p<0.0001$) on PLB (25.4 ± 1.6 ºC) and TYR (25.4 ± 1.5 ºC) trials, which were significantly different from CON (27.7 ± 1.4 ºC, $p<0.05$), but not from each other. The minimum finger temperature achieved during cold air exposure was significantly lower ($p=0.0073$) on the PLB trial (14.7 ± 1.4 ºC), compared to CON (16.0 ± 2.0 ºC, $p<0.05$); but TYR (15.0 ± 1.4 ºC) was not significantly different from either CON or PLB.

3.2 Cold-Water Cognitive Results

3.2.1 Match-to-Sample

Analyses of correct responses on the match-to-sample memory measure demonstrated that fewer correct responses ($P<0.05$) were made with PLB ($\overline{x}$ =-2.36, SEM = 0.77) compared to TYR ($\overline{x}$ =.14, SEM = 0.80) and CON ($\overline{x}$ =.18, SEM = 0.67).

Analyses of reaction time for hits following a 16 second delay revealed a treatment by time interaction (P<0.05), that is, volunteers responded more quickly with TYR ($\bar{x}$ = -1.11, SEM = 6.13) compared to PLB ($\bar{x}$ = 19.38, SEM = 5.37) following the first cold immersion (Figure 1), but there was no difference in RT following the second immersion (TYR $\bar{x}$ = 12.24, SEM = 9.34; PLB $\bar{x}$ =7.62, SEM = 7.51).

A main effect of treatment (P<0.05) for study time across both delays was also observed. Post hoc analyses revealed that volunteers studied the sample for a shorter amount of time with TYR ($\bar{x}$ = -2.18, SEM = 0.47) than PLB ($\bar{x}$ = -0.80, SEM = 0.34) and CON ($\bar{x}$ = -0.61, SEM = 0.41).

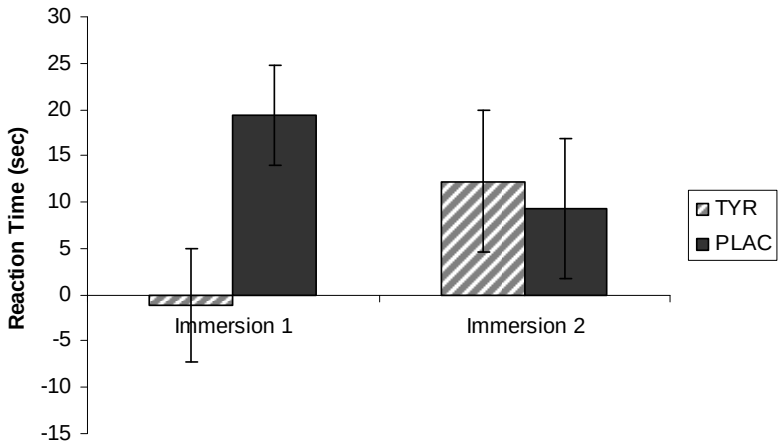

Figure 1: Change in Reaction time (seconds), relative to baseline, for Match-to-Sample following each immersion for tyrosine (TYR) and placebo (PLB) trials.

3.2.2 4-Choice Reaction Time

Analyses of the 4-choice reaction time task revealed a main effect (P<0.01) of temperature on the reaction time measure, such that volunteers responded more rapidly in the CON ($\bar{x}$ = -28.15, SEM = 6.29) than in the cold (TYR $\bar{x}$ =12.91, SEM = 9.34; PLB $\bar{x}$ = 17.38, SEM = 7.63).

Analyses of errors revealed a main effect of temperature for premature errors (P<0.05). Post hoc analyses show that more premature errors were made following cold exposure (TYR $\bar{x}$ = 0.97, SEM = 0.42; PLB $\bar{x}$ = 1.18, SEM = 0.35) than CON ($\bar{x}$ = 0.38, SEM = 0.19). Analyses of errors also showed a main effect for temperature on total errors (p<0.05), such that the total error score was higher following cold exposure (TYR $\bar{x}$ =2.47, SEM = 0.85; PLB $\bar{x}$ =3.29, SEM = 0.90) compared to CON ($\bar{x}$ = 0.74, SEM = 0.58).

3.2.3 Environmental Symptoms Questionnaire

Analyses of the “cold symptom” measure on the ESQ revealed a main effect of temperature (P<0.01), such that volunteers felt more symptoms of cold stress following cold (TYR $\bar{x}$ =1.75, SEM = 0.25; PLB $\bar{x}$ =1.85, SEM = 0.26) compared to CON ($\bar{x}$ = 0.07, SEM= 0.08), with no difference between TYR and PLB. Muscle discomfort was higher (P<0.01) following cold (TYR $\bar{x}$ =1.11, SEM = 0.23; PLB $\bar{x}$ =1.01, SEM = 0.20) vs. CON ($\bar{x}$ = -0.09, SEM= 0.10).

3.2.4 Profile of Mood States

Tension was higher (P<0.01) in the cold (TYR $\bar{x}$ =2.33, SEM = 0.92; PLB $\bar{x}$ =2.07, SEM = 0.58) compared to CON ($\bar{x}$ = -1.76, SEM= 0.65). Depression was also higher (P<0.05) in the cold (TYR $\bar{x}$ =2.37, SEM = 1.16; PLB $\bar{x}$ =2.37, SEM = 0.93) vs. CON ($\bar{x}$ = -0.33, SEM= 0.76). In addition, total mood disturbance, was higher (P<0.01) in the cold (TYR $\bar{x}$ =8.83, SEM = 3.45; PLB $\bar{x}$ =10.1, SEM = .3.54) vs. CON ($\bar{x}$ = -0.37, SEM= 3.07).

Confusion was significantly higher (P<0.01) with PLB ($\bar{x}$ =1.60, SEM= 0.56) compared to CON ($\bar{x}$ = -0.53, SEM= 0.54), but TYR was not significantly different than CON.

3.3 Performance Battery Results

3.3.1 Cognitive Performance

Data from the cognitive performance tests are shown in Table 1. Note that statistical significance is only presented relative to CON because Tukey's HSD post-hoc analysis did not indicate statistical significance between PLB and TYR for any variable. Cold water immersion reduced (P=0.023) the percentage of correct answers on the Match-to-Sample test during the PLB trial for an 18% reduction in performance. Response time was 10% slower (P=0.0084) during the PLB trial for the Addition/Subtraction test. Response time on the Vigilance test improved (P=0.010) on both cold trials by 25%-38%, compared to CON.

	Control	*Placebo*	*Tyrosine*
Match-to-Sample, % Correct	67 ± 15	55 ± 15 *	62 ± 14
Response Time, sec	3.7 ± 0.8	3.3 ± 1.1	3.3 ± 1.1
Complex Reaction Time, % Correct	91 ± 11	89 ± 9.8	89 ± 13.5
Response Time, sec	0.38 ± 0.06	0.40 ± 0.06	0.41 ± 0.07
Addition/Subtraction, % Correct	94 ± 9	93 ± 6	93 ± 7
Response Time, sec	2.0 ± 0.6	2.2 ± 0.8 *	2.1 ± 0.8
Vigilance, % Correct	92 ± 7	96 ± 10	92 ± 8
Response Time, sec	0.08 ± 0.02	0.05 ± 0.03 *	0.06 ± 0.02 *
Grammatical Reasoning, % Correct	82 ± 17	84 ± 15	79 ± 19
Response Time, sec	3.2 ± 1.3	2.9 ± 1.2	3.2 ± 1.6
Repeated Acquisition, % Correct	71 ± 12	70 ± 14	72 ± 12
Response Time, sec	9.9 ± 3.3	9.4 ± 2.7	9.7 ± 2.5

***Tukey's HSD post-hoc, p<0.05 compared to Control.**

Table 1. Cognitive performance during cold-air exposure. Data are presented as mean ± SD.

3.3.2 Psychomotor and Physical Performance

Data from the physical performance tests following resting cold water immersion are shown in Table 2, with marksmanship parameters presented in Table 3. Note again that statistical significance is only presented relative to CON because Tukey's HSD post-hoc analysis did not indicate statistical significance between PLB and TYR for any variable. The only physical performance measures affected by cold were marksmanship (P=0.004), shot group tightness, (P=0.006), vertical shot group tightness (P=0.027), and step test (P<0.0001). Marksmanship performance decreased by 14% on the PLB trial, compared to CON, but there was no significant difference between the TYR and CON for any parameter. On the PLB trial, the poorer marksmanship scores were due to increased shot dispersion and, more specifically, in the vertical direction. Step test performance was reduced by 11% on both PLB and TYR trials, compared to CON.

	Control	*Placebo*	*Tyrosine*
Weapon Assembly, sec	103 ± 25	107 ± 27	114 ± 33
Grip Strength, RH, kg	42 ± 5	41 ± 6	42 ± 5
Grip Endurance, RH, sec	68 ± 16	58 ± 27	56 ± 23
Grip Strength, LH, kg	43 ± 5	42 ± 7	43 ± 5
Grip Endurance, LH, sec	65 ± 22	64 ± 24	61 ± 27
Pull-ups	10 ± 5	9 ± 5	9 ± 5
Steps	53 ± 5	47 ± 6 *	47 ± 7 *
Cycle Test, sec †	1394 ± 158	1449 ± 179	1458 ± 199

***p<0.05 compared to Control. † n=11.**

Table 2. Physical performance following resting cold water immersion. Data are presented as mean ± SD.

	Control	*Placebo*	*Tyrosine*
Distance from Center of Mass, mm	4.7 ± 0.9	5.9 ± 1.2 *	5.3 ± 1.2
Shot Group Tightness, mm^2	59 ± 20	100 ± 46 *	77 ± 30
Horizontal Shot Group Tightness, mm	7.8 ± 2.2	9.4 ± 2.3	8.2 ± 2.2
Vertical Shot Group Tightness, mm	7.6 ± 1.7	10.2 ± 2.8 *	9.1 ± 2.5
Horizontal Deviation, mm	-0.3 ± 1.8	-0.2 ± 2.5	0.1 ± 2.5
Vertical Deviation, mm	-0.7 ± 1.8	-0.2 ± 1.5	-0.7 ± 1.8
Sighting Time, sec	6.4 ± 2.1	7.0 ± 2.4	7.0 ± 2.0

***p<0.05 compared to Control.**

Table 3. Marksmanship performance following resting cold water immersion. Data are presented as mean ± standard deviation.

4.0 DISCUSSION

Physiological stressors such as cold exposure that cause sustained increases in brain catecholamine turnover have been associated with decreases in working memory, and these decrements in performance have previously been shown to be offset by dietary tyrosine administration (35). This was confirmed in the present study, where tyrosine ingestion mitigated the decrease in working memory performance on the Match-to-Sample test following cold water immersion and during cold air exposure that was observed with placebo in subjects with reduced core temperature. However, this study is the first to extend those observations to a more practical demonstration of the effectiveness of tyrosine supplementation for mitigating the cold-induced decrease in psychomotor performance indicated by marksmanship.

As anticipated, exposure to cold stress resulted in impaired performance on multiple cognitive measures. Cold exposure produced decrements on the 4-choice reaction time task. In particular, volunteers responded more slowly and committed a greater number of errors following cold exposure. In addition, performance was impaired on the working memory task at the longest delay interval (16 seconds). These results are consistent with previous work showing that cold stress impairs working memory (2; 34; 35; 39; 40). Previous work suggests that measures of working memory may be particularly susceptible to cold stress and is affected in the early stages of body cooling. Decrements in working memory have been seen with cold exposure resulting in relatively small reductions in core temperature (10) and exposure to cold air that are unlikely to have caused a reduction in core body temperature (35).

Supplementation with tyrosine prior to cold exposure was effective in reducing cold-induced working memory decrements. In fact, administration of tyrosine prior to the cold exposure resulted in matching-to-sample accuracy comparable with that in the thermoneutral condition. This result is consistent with previous work in both animals and humans demonstrating beneficial effects of tyrosine on working memory during stressful conditions (34; 35) and again indicates that tyrosine mitigates working memory impairment when cold-stress specifically affects memory retention, such that accuracy is impaired only at the longest delay interval (35). This study extends previous work by showing that tyrosine is an effective countermeasure against cognitive decrements associated with larger changes in core body temperature, as well as multiple exposures to cold stress in humans. In addition, this study demonstrates that supplementation with the amino acid tyrosine prior to cold exposure reduced the amount of time taken to study the target compared to both the placebo condition and control. This is important because it indicates that when volunteers received tyrosine, they were not only completely protected against cold-induced memory deficits, but they were also able to process information more rapidly. Finally, results from the POMS questionnaire support these observations, as an increased score on the confusion subscale of the POMS following cold is mitigated with tyrosine supplementation.

The beneficial effects of tyrosine supplementation reported in the present study are consistent with the hypothesis that cold stress causes an increase in the firing rate of catecholaminergic neurons and tyrosine supplementation prior to stress increases syntheses of the catecholamines required for optimal working memory function. This hypothesis is supported by studies demonstrating that brain catecholamines, particularly norepinephrine and dopamine, are depleted in certain regions of the brain by exposure to a variety of stressors (8; 17; 36) and that this depletion is associated with decrements in cognitive performance (18; 46). Administration of tyrosine, a precursor for the synthesis of dopamine and norepinephrine (48) has been shown to prevent the stress induced depletion of brain norepinephrine and dopamine in CNS regions associated with working memory (18; 19; 31).

Marksmanship requires cognitive decision-making to determine the optimal time to take a shot, and motor control for physical steadiness required to support the weapon and for control of breathing. Brain catecholamines are important for both cognitive skills and motor control (14; 29; 45), as well as for attention regulation and inhibition of distracting stimuli, both of which would be important for marksmanship skills in a cold environment (4). If brain catecholamine activity during cold exposure is enhanced by supplemental tyrosine, this could contribute to improved marksmanship performance. Support for this mechanism is provided by data in rats, where tyrosine maintained higher brain norepinephrine levels during cold exposure, compared to a placebo trial, and the rats treated with tyrosine exhibited improved behavioral performance (49).

The marksmanship procedure used in the present study has previously been demonstrated to be sensitive to a variety of environmental stressors, including hypoxia (37) and operational stress in a cold-wet environment (38), and was sensitive to the cold exposure in the present study where core temperature was reduced, but upper body tissues were not directly pre-cooled. Other methods of marksmanship evaluation, such as a small arms simulator and use of moving targets, have not demonstrated a decrease in marksmanship performance due to thermal strain (heat or cold) (43). One reason for the discrepant findings regarding cold effects on marksmanship may be the degree of thermal strain induced, since the cold stress used by Tikuisis et al. (43) did not reduce core temperature. In another study, Reading et al. (30) used a similar marksmanship system as the present study to evaluate performance after a 2 h cold air (4ºC) exposure and found no change due to cold exposure, but their findings appear to have been confounded by development of a learning effect in those subjects. Our study observed poorer vertical shot group tightness with body cooling during the PLB trial, suggesting vertical stabilization as a potential intervention to improve marksmanship in the cold through the use of rifle supports such as sandbags. This may be another reason no difference in marksmanship performance was found by Tikuisis et al. (43), since those subjects fired from a prone position using sandbags.

A secondary goal of the present study was to identify which physical performance tasks were sensitive to body cooling. The only physical performance task affected by cold exposure in the present study

was the step test. The impairment in that task was probably due to local tissue cooling produced by cold water immersion in addition to the lowered core temperature. Decreased muscle, nerve, and joint temperatures that occur with direct tissue cooling (e.g., cold water immersion) impair physical performance (13; 16). Although tissue cooling may have been persistent at the beginning of the cycle ergometer test, it is likely that continued activity increased muscle blood flow more than during the step test, probably restoring tissue temperatures to near normal. Physical performance in upper body tasks was unaffected by lowered core temperature.

The tyrosine dose used in the present study (150 mg / kg body weight X 2) is higher than most of the human and animal studies; however, little research has been done to determine an optimal dose. Banderet and Lieberman (6) report plasma tyrosine levels of 109 nmol / ml 150 min after ingestion of 100 mg / kg body weight. Glaeser et al. (15) measured plasma tyrosine concentration for 8 h after both 100 and 150 mg / kg body weight doses, and found plasma tyrosine peaked after 2 h with both doses (154 and 203 nmol / ml, respectively), but only remained elevated after 8 h with the higher dose. For extended cold exposure, the higher dose would seem more appropriate. Badawy and Williams (5) looked at brain dopamine and norepinephrine levels after intraperitoneal tyrosine doses ranging from 5-500 mg / kg body weight, and found the largest elevation in catecholamines at a tyrosine dose of only 20 mg / kg body weight. They suggest that negative feedback mechanisms and / or substrate inhibition of tyrosine hydroxylase could explain the lowered level of catecholamine synthesis despite increased elevation of brain tyrosine. Another question raised by Anisman and Zacharko (3) is how stressors may condition or sensitize the neurotransmitter pathways. For example, repeated exposure to a stressor may increase tyrosine hydroxylase activity, resulting in restored concentration of catecholamines without the need for supplemental tyrosine. Further research is required to produce a dose-response curve for brain catecholamine synthesis following tyrosine supplementation in humans, and to determine whether this relationship is modified by different stressful conditions, by prior exposure to the stressor, or by changes in sensitivity to exogenous tyrosine.

This study supports previous research demonstrating the effectiveness of tyrosine supplementation for mitigating cold-induced cognitive performance decrements such as working memory and extends those findings to include performance on the psychomotor task of marksmanship. The proposed mechanism for this relationship makes the assumption that the reduced brain catecholamine level associated with stressful conditions such as cold exposure is responsible for the degraded cognitive performance, and that elevating tyrosine levels increases tyrosine hydroxylase activity, thereby restoring brain catecholamine levels. However, tyrosine hydroxylase activity may be regulated in a number of ways beyond simply increased tyrosine availability. Future studies could be done to provide further support for the proposed hypothesis. For example, control studies may include administering amino acids that compete with tyrosine uptake, which should reduce the beneficial effects of tyrosine on cognitive performance, or administering amino acids that are precursors to pathways other than catecholamine synthesis. Nonetheless, tyrosine supplementation would appear to be a beneficial countermeasure for cognitive and psychomotor performance during cold strain, and may be expected to be effective under other stressful conditions, including heat stress and altitude exposure. Future studies should also consider whether multi-stressor conditions, such as those Soldiers experience during sustained operations involving environmental stress, physical and mental fatigue, sleep deprivation and caloric restriction, would also benefit from tyrosine supplementation.

DISCLAIMER

Approved for public release; distribution is unlimited. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or reflecting the views of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in Army Regulation 70-25, and the research was conducted in adherence with the provisions of 32

CFR Part 219. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-25 on the use of volunteers in research. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

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